

Reductive Precipitation of Gold by Dissimilatory Fe(III)-Reducing *Bacteria* and *Archaea*

KAZEM KASHEFI, JASON M. TOR, KELLY P. NEVIN, AND DEREK R. LOVLEY*

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

Received 8 January 2001/Accepted 8 April 2001

Studies with a diversity of hyperthermophilic and mesophilic dissimilatory Fe(III)-reducing *Bacteria* and *Archaea* demonstrated that some of these organisms are capable of precipitating gold by reducing Au(III) to Au(0) with hydrogen as the electron donor. These studies suggest that models for the formation of gold deposits in both hydrothermal and cooler environments should consider the possibility that dissimilatory metal-reducing microorganisms can reductively precipitate gold from solution.

A wide diversity of both *Bacteria* and *Archaea* have the ability to transfer electrons to Fe(III) (11, 12, 14). Many of these Fe(III)-reducing microorganisms are also capable of transferring electrons to other metals and metalloids. Microbial reduction of Fe(III) and other metals can influence the fate of metals in aquatic sediments, submerged soils, and the subsurface (10, 11, 13). One of the more geologically significant impacts of microbial metal reduction is the formation of minerals that can be important geological signatures of the activity of metal-reducing microorganisms and, in some instances, that may represent economically important ore deposits. For example, the formation of magnetite during Fe(III) oxide reduction (21) has been considered to be an indication of the activity of Fe(III)-reducing microorganisms in aquatic sediments (4), in the deep, hot biosphere (5), and on Mars (22). The massive magnetite accumulations formed in the Precambrian period, presumably as the result of microbial activity (1, 8, 27), represent an important source of iron ore. It has also been suggested that microbial reduction of U(VI) to U(IV), which precipitates uranium from solution (19), might account for the formation of some uranium ores (6, 17).

Like uranium, gold is soluble in the oxidized form, Au(III), but the reduced form of gold, Au(0), is insoluble (23). The finding that addition of Au(III) to cell suspensions of *Geobacter metallireducens* oxidized *c*-type cytochromes, which are thought to be involved in electron transport to metals (15), suggested that this dissimilatory metal reducer might be able to transfer electrons to Au(III) (9). Furthermore, the *c*₃-cytochrome of *Desulfovibrio vulgaris*, known to be involved in the reduction of U(VI) (20) and Cr(VI) (18), can also transfer electrons to Au(III) (9). However, the possibility that whole cells of either *G. metallireducens*, *D. vulgaris*, or other dissimilatory Fe(III)-reducing microorganisms reduced Au(III) was not further evaluated.

In order to determine the potential for dissimilatory Fe(III)-reducing microorganisms to precipitate gold from solution, the following organisms were cultured (800 ml) in 1-liter bottles, using strict anaerobic techniques as previously described (16,

26; K. Kashefi, J. M. Tor, D. E. Holmes, C. V. Gaw Van Praugh, A.-L. Reysenbach, and D. R. Lovley, submitted for publication): *Pyrobaculum islandicum* (DSM 4184), *Pyrococcus furiosus* (DSM 3638), *Archaeoglobus fulgidus* (DSM 4304), *Ferroglobus placidus* (DSM 10642), *Thermotoga maritima* (DSM 3109), *Pyrobaculum aerophilum* (DSM 7523), *Shewanella algae* strain BRY (ATCC 51181), *G. metallireducens* (ATCC 53774), *Geobacter sulfurreducens* (ATCC 51573), *Desulfuromonas palmitatis* (ATCC 51701), *Geovibrio ferrireducens* (ATCC 51996), *Geothrix fermentans* (ATCC 700665), *D. vulgaris* (ATCC 35115), “*Desulfitobacterium metallireducens*” (laboratory culture collection), and the acetate-oxidizing hyperthermophile strain 234 (laboratory culture collection). Cell suspensions were prepared as previously described (26). Briefly, cells were harvested anaerobically by centrifugation, resuspended in 80 ml of anaerobic bicarbonate buffer under N₂-CO₂ (80:20, vol/vol), and repelleted by centrifugation. This procedure was repeated, and then the cells were resuspended in 8 ml of the bicarbonate buffer under N₂-CO₂ (80:20, vol/vol). An aliquot of cell suspension (ca. 0.1 to 0.2 ml) that provided a final concentration of ca. 0.025 mg of cell protein per ml was added to Au(III)-amended bicarbonate buffer (10 ml; the pH was 7.0 for all organisms except *P. islandicum*, for which it was 6.0 to 6.2). The gas phase was H₂-CO₂ (80:20, vol/vol) when hydrogen was provided as the electron donor or N₂-CO₂ (80:20, vol/vol) when lactate or acetate was the electron donor. Au(III) was provided as gold chloride (HAuCl₄ · 3H₂O; Sigma, St. Louis, Mo.). Incubations were in the dark at, or close to, the organism's optimal growth temperature.

In cell suspension studies, the concentration of Au(III) was measured in filtered (0.2-μm pore diameter) samples injected onto a Dionex DX-500 ion chromatograph. Au(III) was separated on a Dionex IonPac AS5 column with 300 mM NaClO₄-50 mM HCl as the eluent and was detected by absorbance at 215 nm (24; Determination of metal cyanides, application note 55, Dionex Corp., Sunnyvale, Calif.).

Initial studies were conducted with the hyperthermophile *P. islandicum*. The addition of *P. islandicum* to Au(III)-containing bicarbonate buffer in which hydrogen was provided as an electron donor resulted in the rapid formation of purple, colloidal Au(0) at 100°C (Fig. 1). This was associated with a rapid loss of Au(III) from solution (Fig. 2). In contrast, there was no loss of Au(III) when no electron donor was provided or if the

* Corresponding author. Mailing address: Department of Microbiology, University of Massachusetts, 203 Morrill Science Center IV, Amherst, MA 01003. Phone: (413) 545-9651. Fax: (413) 545-1578. E-mail: dlovley@microbio.umass.edu.

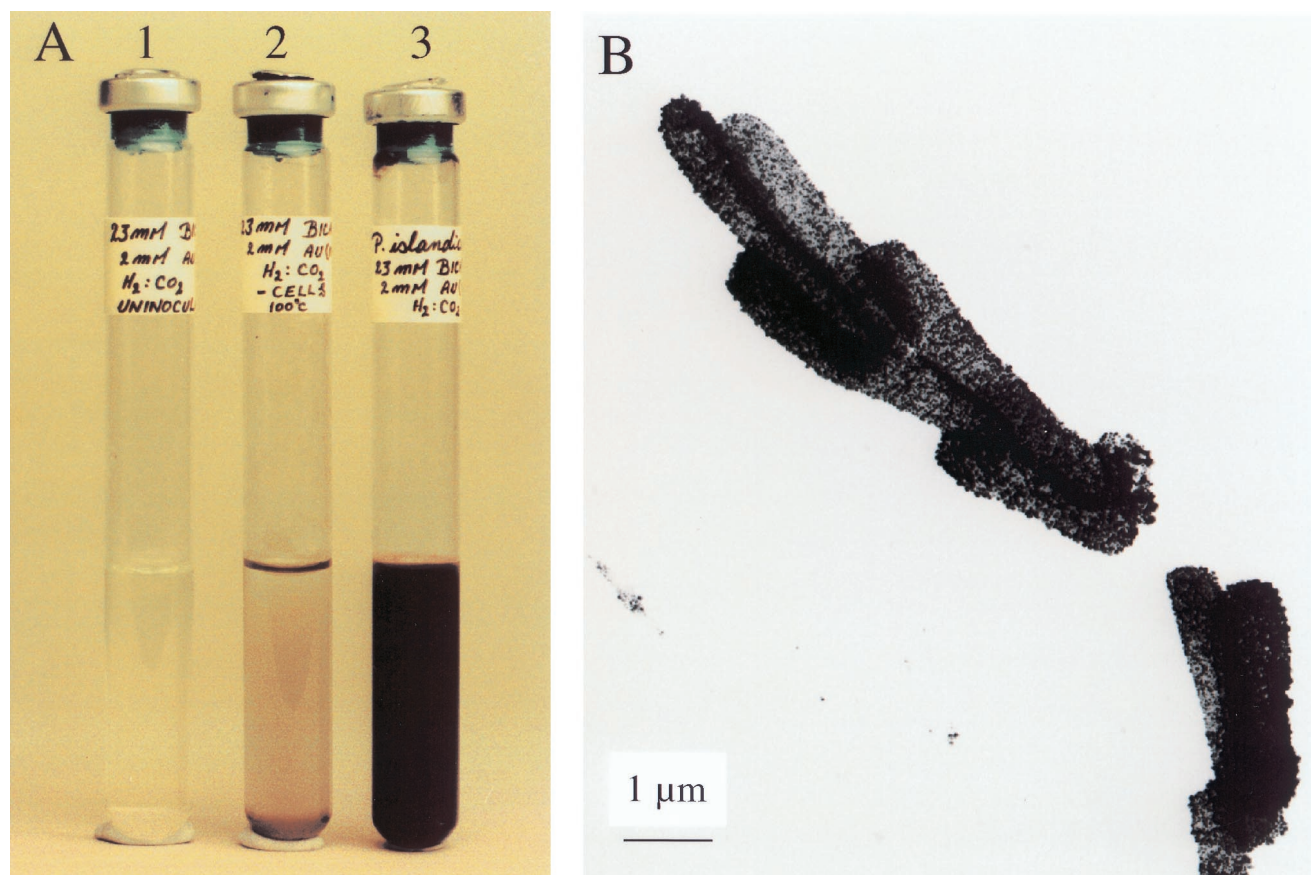


FIG. 1. (A) Visually apparent formation of colloidal gold as the result of Au(III) reduction by *P. islandicum* at 100°C. Tube 1 contains medium prior to incubation at 100°C, tube 2 contains medium incubated at 100°C without cells added, and tube 3 contains medium incubated at 100°C with *P. islandicum* added. (B) Transmission electron micrograph of gold precipitate associated with the cells as the result of Au(III) reduction.

cells were incubated at 30°C. Au(0) precipitated extracellularly with much of the Au(0) attached to the external surface of the cells (Fig. 1). *P. aerophilum*, which is closely related to *P. islandicum*, did not reduce Au(III) (data not shown), even though this organism does have the ability to reduce Fe(III) (6). Some other H₂-oxidizing Fe(III)-reducing hyperthermophilic *Archaea*, including *P. furiosus* and strain 234, did reduce Au(III) with H₂ as the electron donor, precipitating Au(0) extracellularly (Fig. 3). However, *A. fulgidus* and *F. placidus*, the closest known relatives of strain 234 (Kashefi et al., submitted), did not reduce Au(III).

In order to determine if hyperthermophilic Fe(III)-reducing *Bacteria* might also reduce Au(III), studies were conducted with *T. maritima*. Like *P. islandicum*, *T. maritima* reduced Au(III) when H₂ was provided as the electron donor (Fig. 2). *T. maritima*, however, did not reduce Au(III) in the absence of H₂ or when cells were incubated with H₂ at 30°C. The Au(0) that *T. maritima* produced was extracellular (Fig. 3).

The mesophilic Fe(III)-reducing bacterium *S. algae* also had the ability to reduce Au(III) with H₂ as the electron donor (Fig. 2). *S. algae* precipitated Au(0) extracellularly (Fig. 3). Lactate, an alternative electron donor for Fe(III) reduction by *S. algae* (2), did not support Au(III) reduction even though the cells that were used in these studies had been grown with lactate as the electron donor (Fig. 2). In a similar manner, cells

of lactate-grown *D. vulgaris* reduced Au(III) with hydrogen but not with lactate (data not shown). The mesophile *G. ferrireducens* also reduced Au(III), with H₂ as the electron donor, but in contrast to the findings for all of the other organisms examined, Au(0) was precipitated within the periplasmic space (Fig. 3). The other mesophilic, Fe(III)-reducing microorganisms that were evaluated, including the two *Geobacter* species, *D. palmitatis*, and *G. fermentans*, did not reduce Au(III) when either lactate or acetate was used as the electron donor. Furthermore, "*D. metallireducens*" did not reduce Au(III) with lactate or H₂ as the electron donor, while *G. sulfurreducens* did not reduce Au(III) with acetate or H₂ as the electron donor.

The Au(III) reduction reported here appears to be an enzymatically catalyzed reaction which is dependent upon the presence of a specific electron donor, hydrogen. Cells did not reduce Au(III) in the absence of hydrogen or when the metabolic activity of the cells was inhibited by heat treatment or incubation at a temperature too low for the cells to be metabolically active. Only select Fe(III)-reducing microorganisms had the ability to reduce Au(III). Furthermore, in some instances, Fe(III)-reducing microorganisms closely related to Fe(III) reducers that could reduce Au(III) did not reduce Au(III). These results suggest that those Fe(III)-reducing microorganisms that can reduce Au(III) have a specific mechanism for Au(III) reduction that is distinct from the mechanism

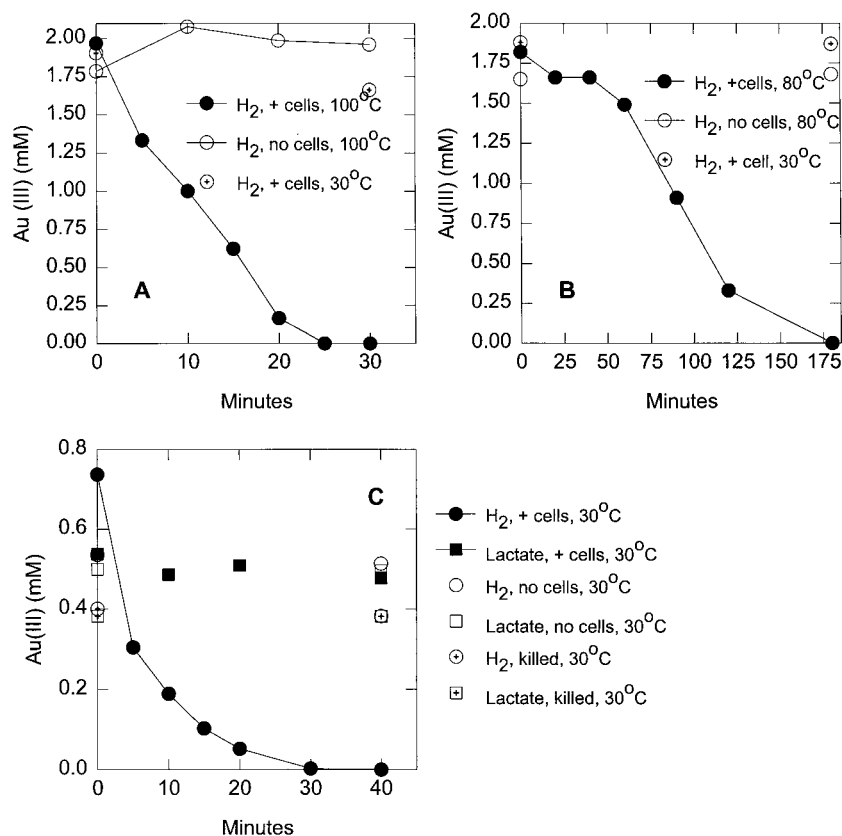


FIG. 2. Hydrogen-dependent Au(III) reduction in Fe(III)-reducing microorganisms. (A) *P. islandicum*; (B) *T. maritima*; (C) *S. algae*.

for Fe(III) reduction. The finding that *S. algae* reduced Au(III) with hydrogen as the electron donor but did not reduce Au(III) when lactate was provided suggests that a hydrogenase is involved in Au(III) reduction. It may be that specific hydrogenases directly reduce Au(III), as has been suggested for Tc(VII) reduction in some organisms (7). However, this hypothesis requires further examination.

The Au(III) reductase of most of the Fe(III) reducers capable of Au(III) reduction appears to be located near the outer cell surface, because Au(0) was precipitated extracellularly. It is assumed that Fe(III)-reducing microorganisms have one or more electron carriers capable of transferring electrons to extracellular electron acceptors, because these organisms can reduce compounds that are unlikely to pass through the outer membrane, such as insoluble Fe(III) oxides and humic substances (11, 12). *G. ferrireducens* precipitated Au(0) in the periplasmic space, suggesting that in contrast to the other Au(III) reducers, the Au(III) reductase in *G. ferrireducens* was localized in the periplasm. Alternatively, the Au(III) reductase in any of these organisms could be located within the cytoplasm, but this would require a mechanism for exporting insoluble Au(0).

Attempts to grow the Fe(III)-reducing microorganisms with Au(III) as the sole electron acceptor were unsuccessful. This might, in some instances, have been the result of Au(III) toxicity. However, strain 234 and *P. islandicum* readily grew in the presence of as much as 1.5 mM Au(III) when Fe(III) oxide

(100 mmol/liter) was also provided as an electron acceptor. This result suggests that at least in the case of these organisms, the lack of growth with Au(III) as the electron acceptor is due to a lack of an energy-conserving electron transport chain coupled to Au(III) reduction rather than to Au(III) toxicity.

The mechanism for the reductive precipitation of Au(0) by Fe(III)-reducing microorganisms appears to be significantly different from previously described microbial mechanisms for the accumulation of Au(0). A number of aerobic microorganisms adsorb Au(III) on the cell surface, with the subsequent reduction of the adsorbed Au(III) to Au(0), through poorly understood mechanisms (3, 23, 25). However, the anaerobic Fe(III)-reducing microorganisms that reduced Au(III) did not appear to have a significant capacity for adsorption of Au(III) prior to reduction, because there was no loss of Au(III) from solution in the absence of hydrogen or when cells were incubated at temperatures that inhibited metabolism. Thus, the results suggest that the Fe(III)-reducing microorganisms reduced Au(III) prior to, or simultaneously with, the adsorption of gold onto the cell surface.

As recently reviewed (23), it has been suggested that microorganisms may be involved in the formation of gold deposits when soluble Au(III) enters an environment in which microorganisms either alter environmental conditions to promote the precipitation of gold or adsorb Au(III) and then reduce it via unspecified reactions. To our knowledge, it has not previously been suggested that dissimilatory Fe(III)-reducing mi-

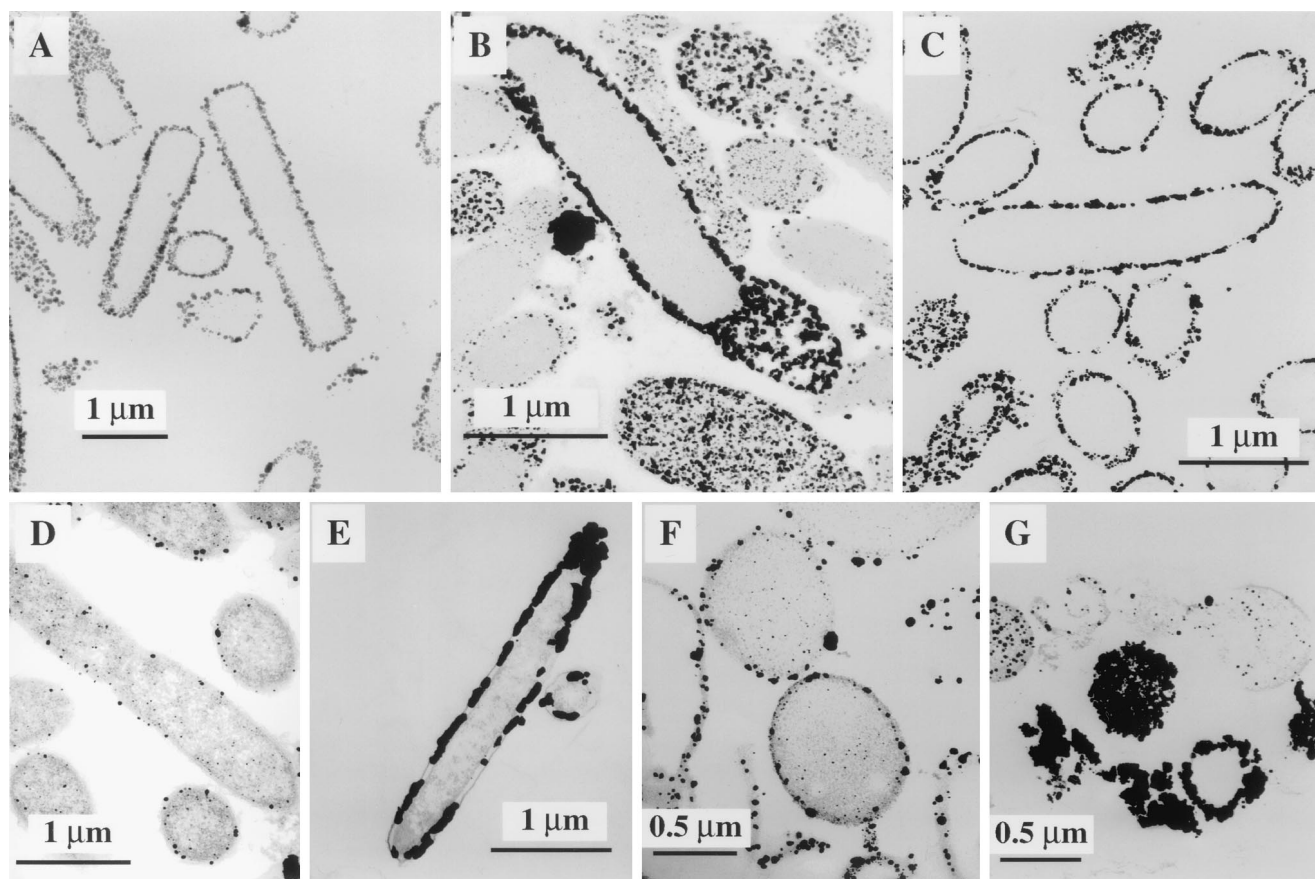


FIG. 3. Transmission electron micrograph of thin sections illustrating gold precipitation associated with Au(III)-reducing microorganisms and lack of gold precipitation with *G. sulfurreducens*. (A) *P. islandicum*; (B) *T. maritima*; (C) *S. algae*; (D) *G. sulfurreducens*; (E) *G. ferrireducens*; (F) *P. furiosus*; (G) strain 234.

croorganisms might contribute to gold ore formation via reduction of Au(III). Although it is not yet possible with the data that are currently available to conclude that Fe(III)-reducing microorganisms have definitely been involved in gold precipitation in any specific environment, Fe(III)-reducing microorganisms are known to be metabolically active in the hot- and moderate-temperature sedimentary environments in which gold deposits have been recovered. The fact that these organisms do not appear to conserve energy to support growth from Au(III) reduction is not likely to be a significant factor in their contribution to the precipitation of Au(III), because these organisms can maintain themselves via the reduction of other electron acceptors that are expected to be abundant in the subsurface, such as Fe(III) and S^0 .

In summary, this study expands the range of metals which dissimilatory Fe(III)-reducing microorganisms are known to reduce. Furthermore, these results provide a previously unrecognized mechanism for the reductive precipitation of gold in subsurface environments that should be considered in models for the formation of gold deposits.

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